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Multiple, temporal-specific roles for HNF6 in pancreatic endocrine and ductal differentiation

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This study is dedicated to the memory of the late Robert H. Costa, Ph.D. without whom this project would not have been possible.

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ABSTRACT

Within the developing pancreas Hepatic Nuclear Factor 6 (HNF6) directly activates the pro-endocrine transcription factor, *Ngn3*. *HNF6* and *Ngn3* are each essential for endocrine differentiation and *HNF6* is also required for embryonic duct development. Most *HNF6*$^{-/-}$ animals die as neonates, making it difficult to study later aspects of HNF6 function. Here, we describe, using conditional gene inactivation, that HNF6 has specific functions at different developmental stages in different pancreatic lineages. Loss of *HNF6* from *Ngn3*-expressing cells (HNF6$^{\Delta endo}$) resulted in fewer multipotent progenitor cells entering the endocrine lineage, but had no effect on β cell terminal differentiation. Early, pancreas-wide *HNF6* inactivation (HNF6$^{\Delta panc}$) resulted in endocrine and ductal defects similar to those described for *HNF6* global inactivation. However, all HNF6$^{\Delta panc}$ animals survived to adulthood. HNF6$^{\Delta panc}$ pancreata displayed increased ductal cell proliferation and metaplasia, as well as characteristics of pancreatitis, including up-regulation of CTGF, MMP7, and p8/Nupr1. Pancreatitis was most likely caused by defects in ductal primary cilia. In addition, expression of Prox1, a known regulator of pancreas development, was decreased in HNF6$^{\Delta panc}$ pancreata. These data confirm that HNF6 has both early and late functions in the developing pancreas and is essential for maintenance of *Ngn3* expression and proper pancreatic duct morphology.

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1. Introduction

Recent advances in experimental techniques allow for more precise control over gene expression revealing that temporal regulation of specific transcription factors is crucial to allocating the correct number of pancreatic progenitor cells to specific pancreatic lineages (Hale et al., 2005; Harmon et al., 2004; Johansson et al., 2007; Schwitzgebel et al., 2000). Understanding the temporal and spatial regulation of these transcription factors is crucial to delineating the molecular mechanisms controlling pancreatic lineage allocation and organogenesis.

The exocrine pancreas, which produces and transports digestive enzymes to the rostral duodenum, is composed of acinar and ductal epithelial cells and accounts for ~98% of adult pancreatic mass. The endocrine pancreas occupies the remaining 2% of pancreatic mass, and is subdivided into five hormone-producing cell types: glucagon-secreting α cells, insulin-secreting β cells, somatostatin-secreting δ cells, pancreatic polypeptide-secreting PP cells, and ghrelin-secreting ε cells (Prado et al., 2004). Endocrine differentiation occurs in two waves: primary transition (pre-e13.5) insulin-expressing and glucagon-expressing cells are not thought to contribute to mature islets, while endocrine cells differentiating during the secondary transition (e13.5–e16.5) will give rise to mature islets (Jensen, 2004; Pictet et al., 1972).

The *H*epatic *N*uclear *F*actor 6 (HNF6) homeodomain-containing transcription factor is an important regulator of endocrine development. HNF6 was first identified in the liver, and 75% of animals with a global HNF6 deletion (HNF6$^{-/-}$) die within the first 10 days after birth due to liver defects (Jacquemin et al., 2000). HNF6 is expressed early in pancreatogenesis in all endodermally-derived cells, but is not detected in differentiated endocrine cells at late-gestation (Landry et al., 1997; Rausa et al., 1997). *HNF6* null embryos show impaired endocrine differentiation and perturbed duct morphogenesis during embryogenesis (Jacquemin et al., 2000; Pierreux et al., 2006). HNF6 acts upstream of the critical pancreatic and β cell transcription factor, pancreas/duodenum homeobox-1 (Pdx1) (Jacquemin et al., 2003) and activates expression of the pro-endocrine gene *Neurogenin 3* (*Ngn3*) (Jacquemin et al., 2000). Ngn3 is the earliest known marker of specified endocrine cells (Gradwohl et al., 2000; Jensen et al., 2000; Schwitzgebel et al., 2000). Global deletion of *HNF6* causes a dramatic down-regulation in *Ngn3* expression, and a concomitant decrease in endocrine cells (Jacquemin et al., 2000), while over-expression of HNF6 specifically in the endocrine lineage results in an increase in NGN3-positive cells (Wilding Crawford et al., 2008). Mice null for *Ngn3* also lack hormone-expressing cells (Gradwohl et al., 2000), and lineage tracing experiments have shown that all islet endocrine cells are derived from a *Ngn3*-expressing progenitor (Gu et al., 2002; Schonhoff et al., 2004). Transgenic expression of *Ngn3* demonstrates that it is sufficient to direct pancreatic progenitors to all endocrine cell types (Johansson et al., 2007).

In this study, we generated mice in which *HNF6* is inactivated throughout the *pdx1* domain (HNF6$^{\Delta panc}$) to further characterize the specific roles of HNF6 in pancreas development and postnatal pancreas function. Similar endocrine defects to those described in the global *HNF6* null animals (Jacquemin et al., 2000) were observed in HNF6$^{\Delta panc}$ animals. To determine whether HNF6 plays additional roles in endocrine differentiation subsequent to *Ngn3* activation, we first clarified the expression of HNF6 protein within the developing pancreas. Based on its lack of expression even in early hormone-positive cells, we hypothesized that HNF6 is required only to initiate endocrine specification. To test this hypothesis, *HNF6* was inactivated specifically in cells that activated *Ngn3* (HNF6$^{\Delta endo}$). Loss of *HNF6* from *Ngn3*-expressing cells did not affect β cell function or glucose homeostasis suggesting that HNF6 is dispensable for later events of endocrine differentiation. Lineage tracing analyses revealed, however, that a subset of *Ngn3*-expressing cells that lost *HNF6* became incorporated into exocrine tissue thereby indicating that activation of *Ngn3* does not irreversibly commit a cell to the endocrine lineage. HNF6 may then function to allow Ngn3 to reach a level within the pro-endocrine compartment that ensures faithful commitment of cells to the endocrine lineage.

In addition to defects in endocrine development, HNF6$^{\Delta panc}$ embryos showed defects in duct development similar to those observed in HNF6$^{-/-}$ embryos (Pierreux et al., 2006). HNF6$^{\Delta panc}$ animals all survived past weaning, allowing us to determine the consequences of HNF6 inactivation in the ductal epithelium. HNF6$^{\Delta panc}$ adult pancreata displayed morphological changes consistent with pancreatitis, an inflammatory process in which pancreatic enzymes autodigest the pancreas. Several molecular markers of pancreatitis, including connective tissue growth factor (CTGF), metalloproteinase-7 (MMP7), and p8/Nupr1 were elevated in HNF6$^{\Delta panc}$ mice. Moreover, these mice exhibited defects in primary cilia of pancreatic epithelial cells, a finding previously associated with increased cyst formation, pancreatitis, and ductal epithelial proliferation (Cano et al., 2006; Pugacheva et al., 2007). Pancreatitis significantly elevates the risk of pancreatic cancer in humans (Lowenfels et al., 1993), and indeed, we observed preneoplastic lesions in HNF6$^{\Delta panc}$ pancreata including squamous cell metaplasia and acinar-to-ductal metaplasia.

2. Results

2.1. *HNF6 is rapidly down-regulated as pancreatic progenitors are specified to the endocrine lineage*

To determine the function of HNF6 in pancreas development subsequent to specification and early bud outgrowth, *HNF6* was inactivated by interbreeding of *HNF6*$^{flox/flox}$ mice with *pdx1*-Cre^{early} transgenic mice (Gu et al., 2002). *pdx1*-Cre^{early} mediates recombination throughout the endogenous *pdx1* domain: antral stomach, rostral duodenum, and entire pancreatic epithelium at e9.5 (Zhang et al., 2006). In *HNF6*$^{flox/flox}$; *pdx1*-Cre^{early} (HNF6$^{\Delta panc}$) mice, Cre recombinase mediates the deletion of the entire cut-domain, generating a null allele (see Section 4 and Fig. 1A). HNF6$^{\Delta panc}$ mice were born at the expected frequency and survived past weaning. In control pancreatic buds at e11.5, HNF6 was detected in all Pdx1-positive cells (Fig. 1Ba), but the majority of Pdx1-positive cells were HNF6 negative in HNF6$^{\Delta panc}$ animals (Fig. 1Bb). By e13.5, HNF6 protein was no longer detectable in HNF6$^{\Delta panc}$